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HEMATOLOGIC CHANGES DURING AND FOLLOWING CARDIOPULMONARY BYPASS AND THEIR RELATIONSHIP TO NON-SURGICAL BLOOD LOSS. I. PLATELET FUNCTION AND THE BLEEDING TIME

BY

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The changes in platelet parameters and the bleeding time during and following cardiopulmonary bypass (CPB) and their relationships to non-surgical blood loss following the administration of protamine were investigated in 85 adult male patients undergoing coronary artery and valvular heart surgery. The bleeding time was determined preoperatively, and 2, 24, 48, and 72 hours postoperatively. The blood shed from the site of the bleeding time determination was assayed for thromboxane B2 (shed blood TxB2) and 6-keto-prostaglandin F-1-alpha (shed blood 6-keto) levels. Blood samples were

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obtained at the above time points and 20 minutes into CPB. Temperatures were measured at the skin adjacent to the template bleeding time site, and in the subcutaneous tissue of the sternotomy wound. The bleeding time 2 hours post-CPB was markedly prolonged and was associated with a marked reduction in the shed blood TxB2. The subsequent reduction in the bleeding time and the increase in the shed blood TxB2 occurred primarily between 2 and 24 hours post-CPB and was associated with a significant increase in MPV, indicative of the release of new large platelets into the peripheral circulation. The bleeding time at 2 hours post-CPB correlated significantly with the blood loss during the initial 4-hour post-CPB period and was determined primarily by the preoperative bleeding time, the duration of CPB, and the skin temperature. Likewise, blood loss during the initial 4-hour post-CPB period correlated during CPB, the duration of CPB, and the bleeding time, hematocrit, and midsternotomy wound temperature at the 2-hour post-CPB period. These data established a direct relationship between the bleeding time and the non-surgical blood loss following CPB and suggested that the postoperative improvement in bleeding time was due to new platelet formation and, possibly, rewarming.

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HEMATOLOGIC CHANGES DURING AND FOLLOWING CARDIOPULMONARY BYPASS AND THEIR RELATIONSHIP TO NON-SURGICAL BLOOD LOSS

I. Platelet Function and the Bleeding Time

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ULTRAMINI-ABSTRACT

In 85 patients undergoing cardiopulmonary bypass, the postoperative increase in the bleeding time was associatied with a reduction in shed blood thromboxane B2 and related directly to postoperative blood loss, the duration of CPB, and the skin temperature. Postoperative improvement in platelet function was due to new platelet formation and, possibly, rewarming.

ABSTRACT

The changes in platelet parameters and the bleeding time during and following cardiopulmonary bypass (CPB) and their relationships to non-surgical blood loss following the administration of protamine were investigated in 85 adult male patients undergoing coronary artery and valvular heart surgery. The bleeding time was determined preoperatively, and 2, 24, 48, and 72 hours postoperatively. The blood shed from the site of the bleeding time determination was assayed for thromboxane B2 (shed blood TxB2) and 6-keto-prostaglandin F-1-alpha (shed blood 6-keto) levels. Blood samples were obtained at the above time points and 20 minutes into CPB. Temperatures were measured at the skin adjacent to the template bleeding time site, and in the subcutaneous tissue of the sternotomy wound. The bleeding time 2 hours post-CPB was markedly prolonged and was associated with a marked reduction in the shed blood TxB2. The subsequent reduction in the bleeding time and the increase in the shed blood TxB2 occurred primarily between 2 and 24 hours post-CPB and was associated with a significant increase in MPV, indicative of the release of new large platelets into the peripheral circulation. The bleeding time at 2 hours post-CPB correlated significantly with the blood loss during the initial 4-hour post-CPB period and was determined primarily by the preoperative bleeding time, the duration of CPB, and the skin temperature. Likewise, blood loss during the initial 4-hour post-CPB period correlated

during CPB, the duration of CPB, and the bleeding time, hematocrit, and midsternotomy wound temperature at the 2-hour post-CPB period. These data established a direct relationship between the bleeding time and the non-surgical blood loss following CPB and suggested that the postoperative improvement in bleeding time was due to new platelet formation and, possibly, rewarming.

INTRODUCTION

Abnormal bleeding is a well known complication of cardiac surgery which may lead to reoperation in a finite percentage of patients (1, 2). Although a number of alterations in hemostasis have been reported during cardiopulmonary bypass (1, 3, 4, 5), it is now becoming apparent that the main defect in hemostasis following the institution of cardiopulmonary bypass is a defect in the formation of the platelet plug manifested by an extension of the template bleeding time (1, 3, 6-9). Thrombocytopenia is commonly associated with cardiopulmonary bypass, but usually it is not severe enough to account for the extension of bleeding time observed during and following cardiopulmonary bypass. Because non-surgical bleeding following cardiopulmonary bypass continues to be a major source of frustration to the cardiac surgeon, a study was designed to characterize in adult patients the hematologic changes which occur during and following cardiopulmonary bypass and to examine the relationships between these changes, the template bleeding time and the extent of non-surgical blood loss. In this communication, we report the alterations in platelet parameters associated with cardiopulmonary bypass and the relationships among the platelet parameters, the bleeding time, and blood loss. The changes in oncotic, opsonic and coagulation proteins and their respective relationships to non-surgical blood loss are reported in a separate communication (10).

MATERIALS AND METHODS

One hundred consenting patients (98 males and 2 females) undergoing open-heart surgery at the West Roxbury Department of Veterans Affairs Medical Center signed informed consent forms before being entered into this study. Patient age ranged from 32 to 77 years, averaging 58.7 years. None of the patients had received antiplatelet therapy in the 10-day pre-operative period. The operations, performed by two surgeons using identical techniques, included the following: 65 isolated coronary artery bypass grafting procedures (CABG), 10 single valve replacements, 9 single valve replacements with CABG, and 1 double valve replacement.

Pre-anesthetic medications included intramuscular Innovar (2 ml) and Atropine (0.4-0.6 mg). Anesthesia was induced with Fentanyl and maintained with a combination of Fentanyl, muscle relaxants, and either Halothane or Isoflurane. Patients were placed on extracorporeal circulation using standard techniques and cooled systemically to a temperature range of 30-22 C depending on the complexity of the surgical procedure. The pump was primed with lactated Ringer's solution. During the period of aortic clamping, the heart was selectively cooled with a cardioplegic solution and with topical ice slush to a temperature range of 8-15 C. In all the patients, the extracorporeal circuit was identical except for the type of oxygenator which was prospectively randomized between the Bentley Bos 10S bubble oxygenator (American Bentley, Irvine, CA) and the Terumo Capiox II hollow fiber membrane oxygenator (Terumo Corporation, Piscataway, NJ).

Heparin was administered prior to institution of cardiopulmonary bypass in an initial dose of 3 mg/kg body weight; subsequent dosages were administered according to the level of the Activated Clotting Time (ACT) which was maintained above 400 seconds throughout the extracorporeal circulation period. At the end of this period heparin was neutralized with protamine sulfate given in a ratio of 0.5 mg protamine to 1.0 mg for the initial heparin dose and 1.0 mg protamine to 1.0 mg for all subsequent heparin doses. Heparin neutralization was also monitored by the ACT. The systemic temperature was measured intraoperatively by an esophageal thermometer and postoperatively by a rectal thermometer.

Measurement of postoperative blood loss was started intraoperatively when the ACT normalized after the administration of the initial protamine dose. It was achieved by collecting all the blood aspirated from the surgical field and by weighing all the surgical sponges. Postoperatively, an accurate record was kept of the mediastinal drainage until the mediastinal tube was removed, usually on the first or second postoperative day. Fifteen patients in whom excessive bleeding occurred from a specific surgical site were identified and excluded from the data analysis. Detailed records were also kept of all the blood products transfused. Platelet concentrates were prepared from units of blood collected into the citrate-phosphate-dextrose (CPD) anticoagulant. The platelet concentrates were stored in 60 ml of plasma at room temperatures with agitation for up to 5

days. On the day of the transfusion, 3-10 units of ABO-compatible platelet concentrates were pooled and transfused.

Bleeding times were determined before the induction of anesthesia and again at 2, 24, 48, and 72 hours following the cardiopulmonary bypass procedure. Blood shed from the template bleeding time site (Figure 1) was assayed for (1) thromboxane B2, the stable metabolite of thromboxane A2, which will be referred to henceforth as "shed blood thromboxane B2", and (2) 6-keto prostaglandin F-1-alpha (6-keto PGF1a), the stable metabolite of prostacyclin, which will be referred to henceforth as "shed blood 6-keto". Blood samples were also withdrawn before induction of anesthesia, 20 minutes after institution of cardiopulmonary bypass, and 2, 24, 48, and 72 hours after cardiopulmonary bypass. These samples were analyzed to determine a total of 30 hematologic parameters. This communication reports only on the 12 parameters listed in Table 2. Skin temperature from the site of the bleeding time measurement was recorded utilizing a 1 cm diameter thermistor pad (Skin Temperature Sensor, Mon-a-therm, Inc., St. Louis, MO). Median sternotomy wound temperature was measured by a 2 cm long thermistor needle which was inserted subcuticularly through the edge of the wound prior to skin closure. All temperatures were recorded continuously.

Laboratory Procedures

Standard template bleeding times were performed in duplicate using the Simplate II bleeding time module (General Diagnostics, Durham, NC) according to the procedure of Babson and Babson (11).

Each template produced two skin incisions. One template was used for the measurement of duplicate bleeding times and the mean value was recorded. The other template was used to collect the shed blood from the site of two skin incisions. The blood shed from these incisions was aspirated and collected through a blunt needle using a 1 ml syringe containing heparin (1000 U/ml) and 40 lambda of ibuprofen (1.9 mg/ml), (Figure 1). A volume of 0.6 ml of blood was collected into the syringe from the bleeding time site at 30-second intervals and kept on ice until it was centrifuged. Each blood sample was centrifuged at 1650 X g (3000 rpm) in a Sorvall GLC-3 centrifuge for 10 minutes; the plasma was removed and kept frozen at -80 C until measurements of shed blood thromboxane B2 and 6-keto PGF1a levels were made. Blood samples were collected in K_3EDTA anticoagulant for measurements of hemoglobin concentration (using the cyanmethemoglobin technique and a Coulter hemoglobinometer, Coulter Electronics, Edison, NJ), hematocrit value (vol %), white blood cell count, platelet count, and mean platelet volume. The platelet counts were performed by phase microscopy. The platelets were sized using a linear scale on a Coulter ZBI Counter with an H4 Channelyzer attachment (Coulter Electronics, Hialeah, FL) and a 50/60 aperture. Details of these procedures and calibration routines have been reported previously (12). Mean platelet mass (u^3 X 10^8/ml) was calculated from the product of mean platelet volume (u^3) and platelet count (X $10^3/mm^3$). The BTG level was measured as follows using a BTG RIA kit (Amersham Corp., Arlington Heights, IL): Blood was collected in 134 mM EDTA and 15 mM

theophylline (13), stored on wet ice, and centrifuged at 2000 Xg (2800 rpm) in a refrigerated Beckman J-5B centrifuge for 30 minutes at 4 C, after which the top 500 μl of the supernatant was frozen and stored at -80 C until it was thawed for assay.

To measure plasma thromboxane B2 and 6-keto PGF1a, 2 ml of arterial blood was collected into tubes coated with heparin (1000 U/ml, USP) and containing 40 lambda of ibuprofen (1.9 mg/ml). The blood sample was kept on wet ice until it was centrifuged at 1650 X g (3000 rpm) in a Sorvall GLC-3 centrifuge for 10 minutes, after which the plasma was removed and kept frozen at -80 C until it was thawed and assayed. Measurements were also made in the shed blood samples obtained at the bleeding time site using thromboxane B2 (^{125}I) RIA kits and 6-keto PGF1a (^{125}I) RIA kits (New England Nuclear Corp., Boston, MA). The 6-keto PGF1a level was corrected for cross-reactivity of the thromboxane B2 antigen with the antibody to the 6-keto PGF1a antigen as follows: 1.4% of the thromboxane B2 level was subtracted from the 6-keto PGF1a level. The total protein level in the blood sample was measured by the Biuret reactions method of Kingsley et al (14).

Data Analysis

The term "post-CPB" in this manuscript denotes the period starting after the complete administration of the first dose of protamine to reverse the heparin. Data are expressed as mean ± standard error of the mean (SEM). Repeated measures analysis of variance (MANOVA) was used to detect significant changes in a variable throughout the study with respect to time, i.e.,

following bypass. When a significant change in a variable was noted over time by MANOVA, the paired student's t-test was used to identify significant differences between specific time points within a group. The differences in the measured variables between the bubble oxygenator and membrane oxygenator groups, and Group 1 and Group 2 were analyzed by the unpaired student's t-test at discrete time points. To graphically display the relationships of postoperative blood loss and the template bleeding time to other measured variables, the entire patient population was divided into tierciles based on the range of values for the blood loss or the bleeding time in the postoperative period. For blood loss during the initial 4-hour post-CPB period the tiercile levels designated were low: 215-790 ml, n=26; medium: 805-1140 ml, n=26; and high: 1235-2515 ml, n=26. For the bleeding time at two hours post-CPB, the tiercile levels designated were low: 5.1-11.45 min, n=27; medium: 11.51-15.77 min, n=27; and high: 16.2-≥20 min, n=27. The relationships of the postoperative template bleeding time and the blood loss to other variables were univariately analyzed using linear regression, and a significant direct relationship between either the bleeding time or the blood loss and the independent variable was determined at the $p < 0.05$ level for the model. These relationships were also examined in a multivariate analysis where the variables predicting the observed postoperative bleeding time and the observed postoperative blood loss were determined using a stepwise multiple general linear regression model. A variable was considered independently significant ($p < 0.05$ for the variable) if it im-

proved the overall model's ability to explain the observed variability in the blood loss or the bleeding time when added last to the multivariate general linear model (i.e., Type III Sum of Squares). All the statistical analyses were performed with the SAS statistical package (Cary, North Carolina) on an IBM-compatible personal computer.

RESULTS

Membrane vs Bubble Oxygenator

Table 1 compares the characteristics of the patients who were randomized to an extracorporeal circuit with a membrane oxygenator to those of the patients who were randomized to a bubble oxygenator. Both groups of patients were comparable regarding age, type of operation, cardiopulmonary bypass time, cross-clamp time, lowest esophageal temperature during cardiopulmonary bypass, and total postoperative blood loss. No significant differences between the two groups were observed in any of the postoperative parameters investigated. For this reason, type of oxygenator was not entered as a co-variate in the multivariate analysis performed.

Changes in Platelet Function and Other Parameters During and Following Cardiopulmonary Bypass

Changes in platelet function during and following cardiopulmonary bypass were analyzed separately in the 54 patients who underwent isolated coronary artery bypass grafting without receiving any platelet transfusions during their hospitalization

(Group 1), and in the 31 patients who received platelet transfusions during their hospitalization for more complicated cardiac surgical procedures (Group 2). Group 1 had significantly shorter CPB and cross-clamp times and higher esophageal, skin, and wound temperatures when compared to Group 2 (Table 2). Blood loss during the initial 4-hour post-CPB period was also significantly reduced in Group 1, and these patients required fewer units of fresh frozen plasma.

Means $\pm$ standard errors for the hematologic parameters in the two patient groups are shown in Table 3 and in Figures 2-4. Repeated measures analysis of variance (MANOVA) showed that the changes in each parameter with respect to time in the course of the study were highly significant ($p < 0.001$) for all variables.

Changes in Hematocrit and Platelet Parameters (Figure 2, Table 3): There were no differences between the two groups in any of the baseline values of these measurements. The hematocrit fell in both groups from a normal value to an average of 21% ($p = 0.001$) during cardiopulmonary bypass and then rose to around 30% ($p = 0.0001$) and remained at that level for up to 72 hours postoperatively. At the 2-hour post-CPB period, the hematocrit and hemoglobin levels in Group 2 were significantly lower than in Group 1 (Figure 2). The changes in platelet count were similar in both groups. The platelet count in Group 1 fell from a baseline of 206,000/mm^3 preoperatively to 104,000/mm^3 during cardiopulmonary bypass ($p < 0.0001$). The platelet count expected on the basis of hemodilution alone was 116,000/mm^3 in this group. The expected platelet count was significantly higher than the ob-

served platelet count in both groups ($p < 0.0001$), indicating a loss of platelets from the circulation during cardiopulmonary bypass. In both groups, the platelet count remained constant during the 2-hour to 72-hour post-CPB period. In both groups the mean platelet volume fell to a nadir 2 hours after cardiopulmonary bypass ($p < 0.001$) and then gradually rose over the ensuing 3 days. The rise between 2 hours and 72 hours post-CPB was significant ($p < 0.001$) in both groups. In both groups, a significant rise in platelet mass was observed between 2 and 72 hours following cardiopulmonary bypass ($p < 0.001$).

Bleeding Time, Shed Blood Thromboxane B2 and 6-Keto PGF1a Levels (Figure 3, Table 3): Bleeding time was significantly increased 2 hours after cardiopulmonary bypass, and decreased gradually over the 72-hour post-CPB period, with the greatest decrease taking place between 2 and 24 hours post-CPB. The bleeding time was significantly more prolonged in Group 2 than in Group 1 at 2 hours ($p < 0.03$), and at 24 hours ($p < 0.03$) post-CPB. In Group 1 the mean bleeding time was 2.3 minutes ($p < 0.0001$) greater 24 hours post-CPB than preoperatively; in Group 2 the 24-hour post-CPB value was 4.3 minutes ($p < 0.0001$) greater than the preoperative value. By 24 hours postoperatively, the bleeding time had returned to within 1 minute of its preoperative value in 32% of the patients in Group 1 and in 10% of the patients in Group 2. By 72 hours postoperatively, the respective figures were 67% for Group 1 and 58% for Group 2.

A significant ($p < 0.0001$) reduction in shed blood thromboxane B2 was observed 2 hours following cardiopulmonary bypass

when compared to the pre-CPB control in both groups (Figure 3 and Table 3). In both groups, there was a marked rise in shed blood thromboxane B2 ($p < 0.0005$) between 2 and 24 hours post-CPB, associated with a decrease in plasma thromboxane B2 level (see below). At 72 hours post-CPB the shed blood thromboxane B2 level was not statistically different from the preoperative value in either group. An opposite trend was observed in the shed blood 6-keto level which at 2 hours postoperatively was significantly higher than preoperatively ($p < 0.0001$) and then continued to fall, paralleling the changes in plasma 6-keto discussed below. The shed blood 6-keto levels in both groups were higher than the plasma 6-keto levels during the 2-hour to 72-hour post-CPB period. There were no significant differences in the shed blood thromboxane B2 and 6-keto levels between the two groups of patients.

Plasma Beta Thromboglobulin (BTG), Thromboxane B2 and 6-Keto Levels (Figure 4, Table 3): A marked rise in plasma BTG, a platelet-specific protein, was observed in both groups when first measured 20 minutes after institution of cardiopulmonary bypass ($p < 0.0001$); it remained significantly elevated 2 hours after discontinuation of bypass. At the 2-hour post-CPB period, the value was significantly higher in Group 2 than in Group 1 ($p = 0.003$). The significantly elevated BTG level at 2 hours after bypass in the group of patients subjected to complicated cardiac surgery and requiring platelet transfusions can be explained by the length of total bypass time and by the plasma BTG infused with the liquid-preserved platelets. By 24 hours

postoperatively, plasma BTG returned to its pre-CPB level in both groups. The plasma thromboxane B2 levels of the two groups are shown in Figure 4 and Table 3. They were not statistically different between the two groups. The rise in plasma thromboxane B2 level observed during cardiopulmonary bypass did not attain statistical significance, but the decrease in this level following the discontinuation of bypass and throughout the 48-hour post-CPB period was highly significant ($p < 0.0002$) in both groups. A significant rise in plasma thromboxane B2 was observed between 48 and 72 hours; at 72 hours it was no different from baseline. Plasma 6-keto rose significantly ($p < 0.0001$) during cardiopulmonary bypass in both groups (Figure 4, Table 3). From 24 to 72 hours post-CPB, the level was not significantly different from the preoperative level. No significant differences in the plasma 6-keto level were observed between Group 1 and Group 2. The plasma 6-keto levels were significantly lower than the shed blood 6-keto levels during the 2-hour to 72-hour post-CPB period ($p < 0.001$).

Relation of Bleeding Time to Other Parameters

The preoperative bleeding time was related to the bleeding time in the 2-hour post-CPB period, but not to any of the other postoperative variables. Univariate linear regression analysis demonstrated significant direct relationships between the 2-hour post-CPB bleeding time and the preoperative bleeding time ($p=0.007$), the total CPB time ($p=0.05$), and the 4-hour post-CPB period blood loss ($p=0.03$). As shown in Figure 5, the subset of

patients with the highest bleeding time at the 2-hour post-CPB period had a higher preoperative bleeding time, a longer duration of CPB and an increased amount of blood loss; they also had a lower platelet count and a decreased shed blood thromboxane level but these latter two relationships did not attain statistical significance. In the multivariate stepwise linear regression analysis three variables were independently predictive of the bleeding time at the 2-hour post-CPB period: The preoperative bleeding time ($p=0.0007$), the total CPB time ($p=0.03$), and the skin temperature at the site of the determination of the 2-hour post-CPB bleeding time ($p=0.01$). Approximately 25% of the observed variability in the 2-hour post-CPB bleeding time was explained by the multivariate model ($R^2 = 0.26$).

Relations of the Blood Loss During the Initial 4-Hour Post-CPB Period

The rate of blood loss progressively decreased with time in the immediate postoperative period. At 2 hours post-CPB the rate of blood loss averaged 125 $\pm$ 15 ml/hour. By 4 hours post-CPB it decreased to 62 $\pm$ 8 ml/hour. The marked decrease in bleeding time observed between 2 and 24 hours post-CPB was also accompanied by a marked decrease in the rate of blood loss. At 24 hours postoperatively, the blood loss averaged 12 $\pm$ 2 ml/hour. Since most of the post-CPB bleeding occurred in the initial four hours and since this period was most reflected by the measurements which were made at the 2-hour post-CPB time point, the total blood loss during the initial 4-hour post-CPB period was

chosen to represent the blood loss variable in the data analysis. The blood loss during this period did not relate to any of the preoperative variables. Univariate linear regression analysis demonstrated significant direct relationships between the 4-hour post-CPB period blood loss and the total CPB time ($p=0.0001$), the lowest peripheral skin ($p=0.0007$), lowest mid-sternotomy wound ($p=0.02$), and lowest esophageal ($p=0.02$) temperatures reached during CPB, the platelet mass during CPB ($p=0.04$), and the bleeding time ($p=0.03$), midsternotomy wound temperature ($p=0.01$), and hematocrit ($p=0.005$) during the 2-hour post-CPB period. As shown in Figure 6, the subset of patients with the highest blood loss during the 4-hour post-CPB period had a longer total CPB time, a greater fall of esophageal temperature and a lower platelet mass during CPB, and a lower midsternotomy wound temperature and hematocrit during the 2-hour post-CPB period. This same subset of patients also had a more prolonged bleeding time at the 2-hour post-CPB period. In the multivariate stepwise linear regression analysis only the total CPB time ($p=0.0001$) and the hematocrit at 2 hours post-CPB ($p=0.04$) were independently predictive of the extent of the 4-hour post-CPB period blood loss. Approximately 25% of the observed variability in the 4-hour post-CPB period blood loss was explained by the multivariate model ($R^2 = 0.23$).

DISCUSSION

The results of this study support previously published reports showing that a platelet dysfunction occurs after cardiopulmonary bypass and is manifested by a prolongation of the template bleeding time (3, 6-8). Further, our study demonstrated that: (1) There was an association between the platelet dysfunction and decreased production of thromboxane A2 at the site of the bleeding time determination. (2) The bleeding time at the 2-hour postoperative period following cardiopulmonary bypass correlated with the preoperative bleeding time and the 4-hour postoperative blood loss. (3) The bleeding time and the blood loss in the initial 4-hour period post-CPB were related directly to the skin and midsternotomy wound temperatures respectively. (4) The duration of cardiopulmonary bypass was the best predictor of postoperative platelet dysfunction and blood loss.

Platelet Dysfunction During and Following CPB

A marked increase in the bleeding time was observed 2 hours after the discontinuation of CPB (Figure 3), and was more pronounced in complex patients who underwent longer periods of CPB (Group 2). The increased bleeding time occurred at a time when the platelet counts were identical in the two groups of patients (Figure 2) averaging above 100,000/μl of blood. This finding was similar to that reported by Harker et al (3) and indicated a platelet dysfunction since this degree of thrombocytopenia is not expected to affect the bleeding time (15).

The marked increase in plasma BTG observed in our study during and two hours following CPB was also similar to data reported by Harker and associates and by other investigators who demonstrated a marked increase in the plasma platelet-specific proteins, BTG and Factor 4, following the institution of CPB. This is due to platelet activation and release of alpha granule contents (3, 5, 16-19).

The nature of the platelet dysfunction following CPB is not fully understood. In vitro (20) and in vivo (8) studies have demonstrated fragmentation of platelet membranes, degradation of platelet membrane glycoprotein IIb/IIIa complex, and a reduction in the number of fibrinogen receptors of circulating platelets following cardiopulmonary bypass. George et al (9) have also ascribed the prolongation of the bleeding time in cardiac surgical patients to a decrease in platelet surface glycoproteins Ib and IIb. In our study reported here, a reduction in the level of thromboxane B2 was noted in the shed blood collected from the site of a standardized template bleeding time (Figure 2). A decreased shed blood thromboxane B2 level (the stable metabolite of thromboxane A2) was associated with an extension of the bleeding time at the 2 hour post-CPB period. The measurement of thromboxane B2 and 6-keto PGF1a (the stable metabolite of prostacyclin) in the blood shed from the skin at the site of the bleeding time has yielded valuable information regarding the status of platelet function in studies conducted in the baboon and in man (21-23).

The increase in bleeding time post-CPB was followed by a reduction which occurred primarily between the 2- and 24-hour post-CPB period (Figure 3). The bleeding time returned to its prebypass level by 24 hours in 23% of the patients. In 35% of the patients, the bleeding time remained elevated for 72 hours postoperatively. These findings are at variance with the results reported by Harker and associates (3) which showed a return of the bleeding time to normal by 24 hours. The difference between the two studies could be due to the differences in the number and the type of the patients entered into the two studies. A larger number of more complex patients was included in our study. The impact of the complexity of the operative procedure on the bleeding time and its rate of return to baseline level is evident from a comparison of the two patient groups in our study (Table 2). Group 2 had significantly longer cross-clamp and cardiopulmonary times and required platelet transfusions. Consequently, despite the administration of platelet transfusions, the bleeding time was significantly more prolonged in this group at the 2- and 24-hour post-CPB periods than in Group 1 (Figure 2). Furthermore, in Group 1, the less complex group, the bleeding time returned to its pre-CPB value at 24 hours in 32% of the patients in contrast to 10% of the patients in Group 2.

The increase in bleeding time which was observed two hours following cardiopulmonary bypass was accompanied by a significant reduction in the platelet count and in the mean platelet volume compared to baseline. This indicated that larger platelets were selectively removed from the extracorporeal circuit. This

finding was similar to that observed by Laufer et al (24). Furthermore, while the platelet count remained essentially unchanged during the first 3 postoperative days, the bleeding time returned to normal, and the mean platelet volume significantly increased, reflecting the release of new large platelets into the peripheral circulation. The size of platelets has been shown to be directly related to their function (25). Hence, the appearance of new platelets in the peripheral circulation accounts, at least in part, for the restoration to normal of the bleeding time following cardiopulmonary bypass.

In concert with the increase in mean platelet volume between 2 and 72 hours post-CPB, a significant increase in the level of shed blood thromboxane B2 was observed (Figure 3). The observed increase in the shed blood thromboxane B2 during the first 48 postoperative hours was associated with a reduction in plasma thromboxane B2 (Figure 4), eliminating the possibility that plasma thromboxane B2 accounted for the increased level of thromboxane B2 in the shed blood. Thus, the release of new large platelets into the circulation was at least partially responsible for the increased level of thromboxane B2 in the shed blood during the 2- to 72-hour post-CPB period.

Postoperative Blood Loss, the Bleeding Time, and Hypothermia

Blood loss was carefully measured in this study starting immediately after the complete administration of the first dose of protamine intraoperatively. Surgical bleeders were identified prior to performing any data analysis and were excluded from the

analyses since the aim of the study was to assess the coagulopathy precipitated by extracorporeal circulation. The rate of non-surgical postoperative bleeding was highest during the 4-hour post-CPB period. The direct relationship which was observed during this period between the bleeding time and the measured blood loss has not been reported previously. It confirms previous reports which have shown a correlation between the postoperative bleeding time and the volume of blood products administered to patients in the postoperative period (3,6,26). In contrast to all these observations, Burns et al (27) failed to elucidate any relationship between the postoperative bleeding time and the blood loss following cardiac surgery. It was important in making the correlations in our study that we exclude all patients with identifiable surgical sources of blood loss from our analyses.

Although we observed a direct relationship between the 4-hour post-CPB blood loss and a number of intraoperative and postoperative variables, multivariate analysis showed that the duration of CPB and the hematocrit at 2 hours post-CPB were the variables independently predictive of the post-CPB blood loss. The duration of CPB was thus a determinant of a number of variables such as the lowest skin, esophageal, and midsternotomy wound temperatures during CPB, the postoperative bleeding time, and the postoperative midsternotomy wound temperature, all of which correlated univariately with the 4-hour post-CPB blood loss. Studies in anemic and uremic patients not undergoing cardiopulmonary bypass showed a correlation between anemia and a

prolongation of the bleeding time (28-31). Although our study showed a relationship between a low hematocrit and an increase in blood loss, the hematocrit did not correlate with the post-operative bleeding time. Hence, it is possible that the increased blood loss at low hematocrit was due to a dilutional coagulopathy, or that the low hematocrit itself was a result of the increased blood loss.

The independent predictors of the 2-hour post-CPB bleeding time were the preoperative bleeding time, the duration of CPB, and the 2-hour post-CPB skin temperature at the site of the bleeding time determination. This study, thus, identifies a clear predictive role for the preoperative measurement of the bleeding time and suggests that it should be routinely included in the preoperative workup of the cardiac surgical patient. Our studies in the baboon have shown a good correlation between skin hypothermia and the extension of the bleeding time (21). This latter observation, along with the observations made in the current study have prompted our group to address the relationship between the bleeding time and temperature in a separate study of 38 patients undergoing cardiopulmonary bypass (32). All these studies have demonstrated a direct relationship between bleeding time and skin temperature. Hence the bleeding time and skin temperature must be measured simultaneously.

Effect of Oxygenator on Platelet Function

The type of oxygenator used, whether bubble or membrane, did not influence platelet function, a finding similar to that of Edmunds et al (6). The literature is divided on this subject,

although most of the studies do not show a superiority of one type over the other, particularly during short periods of cardiopulmonary bypass. Boonstra et al (33) demonstrated that cardiotomy suction adversely affected platelet function. They suggested that any advantage a membrane oxygenator may have over the bubble oxygenator to maintain platelets during cardiopulmonary bypass may be masked by the cardiotomy suction.

In summary, this study reports a platelet dysfunction during cardiopulmonary bypass surgery which correlated with postoperative blood loss and which was characterized by a prolongation of the bleeding time and a decreased level of thromboxane A2 at the site of the bleeding time measurement. The subsequent reduction of the bleeding time in the postoperative period was due to the release of new platelets into the circulation and to the possible correction of a reversible hypothermia-induced platelet dysfunction. By reducing the length of cardiopulmonary bypass and restoring core and peripheral temperature to normal it should be possible to reduce platelet dysfunction and postoperative blood loss following cardiopulmonary bypass.

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The authors acknowledge the contribution of Mr. Fred Levan who initiated the study to investigate the difference between membrane and bubble oxygenators utilized during cardiopulmonary bypass surgery; Dr. Philip Lavin who provided all the statistical consultation and advice; Mrs. Nancy Healey and Mr. Michael Park who assisted in the editing and preparation of the tables and figures; and Mrs. Donna Kantarges who provided typing and editing services.

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LEGENDS TO FIGURES

Figure 1. Method of collecting the shed blood at the site of the bleeding time determination. The blood pressure cuff is inflated to 40 mm Hg during the bleeding time determination. Blood collected in the tuberculin syringe is placed on ice and assayed for Thromboxane B2 and 6-keto PGF1a.

Figure 2. The hematocrit, platelet count, mean platelet volume, and platelet mass preoperatively, on-bypass, 2, 24, 48, and 72 hours post-CPB in patients subjected to CABG surgery only and not transfused with platelets (Group 1) and all the other patients (Group 2). There were no significant differences between the two groups except for the hematocrit at 2 hours post-CPB (*). See text for the significance of the changes in each parameter over the course of the study.

Figure 3. The bleeding time, the shed blood thromboxane B2 (TxB2) and 6-keto PGF1a (6-keto) levels preoperatively, on-bypass, 2, 24, 48, and 72 hours post-CPB in patients subjected to CABG surgery only and not transfused with platelets (Group 1) and all other patients (Group 2). The bleeding time was significantly (*) more prolonged at 2 and 24 hours post-CPB in Group 2 compared to Group 1. No other significant differences between the two groups were observed. See text for the significance of the changes in each parameter over the course of the study.

Figure 4. : The preoperative bleeding time, the duration of cardiopulmonary bypass (CPB), and the postoperative levels of plasma beta-thromboglobulin (BTG), plasma Thromboxane B2 (TxB2)

and plasma 6-keto PGF1a (6-keto) preoperatively, on-bypass, and 2, 24, 48, and 72 hours post-CPB in patients subjected to CABG surgery only and not transfused with platelets (Group 1) and all other patients (Group 2). Except for the plasma BTG at 2 hours post-op, which was significantly (*) higher in Group 2 than in Group 1, there were no differences between the two patient groups in any of these parameters. See text for the significance of the changes in each parameter over the course of the study.

Figure 5. Preoperative, intraoperative, and postoperative variables in tierciles of the bleeding time at 2 hours post-CPB.

Figure 6. Preoperative, intraoperative, and postoperative variables in tierciles of the blood loss during the initial 4 hours post-CPB.

Table 1: Characteristics of the patients randomized to membrane vs. bubble oxygenator

	Membrane	Bubble	p value
Number of Patients	43	42	
Age (Years)	58 ± 1.3	59 ± 1.3	0.75
Type of Operation (Number of Patients)			
CABG	33	32	0.76
CABG+Valve Replacement	3	6	0.28
Valve Replacement	7	4	0.95
Total Pump Time (Minutes)	146.7 ± 6.8	129.7 ± 9.4	0.15
Total Aortic Clamp Time (Minutes)	62.6 ± 4.8	51.0 ± 4.0	0.07
Lowest Systemic Temperature During CPB (°C)	24.4 ± 0.5	24.6 ± 0.5	0.73
Total Blood Loss (ml)	1948 ± 145	1838 ± 173	0.62

CPB = Cardiopulmonary Bypass
CABG = Coronary Artery Bypass Graft

Table 2: Comparison of patients with isolated CABG who did not receive platelet transfusions (Group 1) and patients with valvular disease and/or CABG who received platelet transfusions (Group 2).

	Group 1	Group 2	p values
Number of Patients	54	31	
Age	58±1	60±1	NS
PROCEDURE			
CABG	54	11	
CABG + Valve	0	9	
AVR	0	6	
MVR	0	4	
AVR+MVR	0	1	
Total Bypass Time	120.0±5.1	168.5±11.5	0.0005
Aortic Clamp Time	44±2	79±7	0.0001
BLOOD PRODUCTS			
Units of Fresh Frozen Plasma	1.2±0.4	3.3±0.6	0.002
Units of Pooled Platelets	0	10.2±1.4	
Units of Packed Cells	4.8±0.5	6.4±0.6	0.04
Units of Whole Blood	1.1±0.2	1.6±0.4	0.26
TEMPERATURES (°C)			
During CPB			
Lowest Esophogeal Temperature	25.1±0.4	23.3±0.5	0.01
Lowest Skin Temperature	26.6±0.3	25.0±0.3	0.0002
Lowest Wound Temperature	22.3±0.5	19.1±0.9	0.001
At 2 Hours Post-CPB			
Skin Temperature	31.3±0.2	31.3±0.2	0.96
Wound Temperature	33.8±0.2	33.3±0.4	0.29
BLOOD LOSS (ml)			
4 Hours Post-Op	929±61	1458±178	0.008
Total Post-Op	1638±96	2333±233	0.009

CPB = Cardiopulmonary Bypass
NS = Not Significant

Table 3: Mean ± S.E.M. for all parameters analyzed in the two patient groups

		PreBP	OnBP	2 Hrs	24 Hrs	48 Hrs	72 Hrs
Hematocrit (%)	Group 1	37.6 ± 0.5	21.0 ± 0.4	33.6 ± 0.6	30.4 ± 0.6	29.4 ± 0.5	29.9 ± 0.6
	Group 2	39.2 ± 0.8	22.3 ± 0.7	31.1 ± 0.8	28.8 ± 0.9	28.5 ± 0.5	28.7 ± 0.6
Hemoglobin (g/dL)	Group 1	13.5 ± 0.2	7.5 ± 0.2	12.3 ± 0.2	11.0 ± 0.2	10.5 ± 0.2	10.6 ± 0.2
	Group 2	14.0 ± 0.4	7.8 ± 0.3	11.3 ± 0.3	10.3 ± 0.3	10.1 ± 0.2	10.2 ± 0.2
Total Protein (g/dL)	Group 1	6.38 ± 0.13	3.23 ± 0.09	4.76 ± 0.12	4.78 ± 0.14	5.01 ± 0.12	5.08 ± 0.13
	Group 2	6.17 ± 0.16	3.23 ± 0.16	4.70 ± 0.12	4.78 ± 0.16	4.90 ± 0.15	5.02 ± 0.22
Bleeding Time (min)	Group 1	7.7 ± 0.3	---	12.9 ± 0.6	10.5 ± 0.5	10.9 ± 0.5	9.5 ± 0.5
	Group 2	8.3 ± 0.6	---	15.2 ± 0.8	12.6 ± 0.7	11.3 ± 0.7	10.2 ± 0.7
Platelet Count	Group 1	206 ± 7	104 ± 5	128 ± 6	123 ± 6	109 ± 6	124 ± 6
($x10^3/mm^3$)	Group 2	198 ± 12	94 ± 8	121 ± 8	119 ± 9	116 ± 12	135 ± 17
Mean Platelet Volume	Group 1	8.34 ± 0.24	7.70 ± 0.2	7.18 ± 0.25	8.03 ± 0.26	8.48 ± 0.26	9.02 ± 0.27
(μ^3)	Group 2	8.24 ± 0.25	7.95 ± 0.33	7.07 ± 0.28	8.16 ± 0.28	8.35 ± 0.30	8.89 ± 0.40
Platelet Mass	Group 1	17.1 ± 0.7	8.02 ± 0.43	9.27 ± 0.60	9.83 ± 0.55	8.92 ± 0.55	10.9 ± 0.6
($\mu^3 x10^8$/mL)	Group 2	16.6 ± 1.1	8.04 ± 0.97	9.10 ± 0.78	9.75 ± 0.86	9.90 ± 0.97	12.1 ± 1.6
Plasma BTG	Group 1	59.3 ± 4.5	285.1 ± 29.0	291.7 ± 39.2	57.9 ± 4.6	58.9 ± 17.9	50.3 ± 6.2
(ng/.1mL)	Group 2	61.1 ± 8.2	275.0 ± 31.4	554.2 ± 73.4	79.5 ± 11.5	73.9 ± 16.6	49.9 ± 6.4
Plasma TxB2	Group 1	88.3 ± 8.8	90.6 ± 4.8	76.2 ± 7.2	59.4 ± 4.3	59.0 ± 4.2	77.1 ± 10.6
(pg/.1mL)	Group 2	86.5 ± 9.5	104.3 ± 8.1	73.0 ± 6.7	61.6 ± 5.4	58.7 ± 5.7	73.9 ± 11.0
Shed Blood TxB2	Group 1	571 ± 43	---	245 ± 30	405 ± 46	457 ± 37	531 ± 46
(pg/.1mL)	Group 2	506 ± 53	---	219 ± 30	423 ± 82	590 ± 113	554 ± 64
Plasma 6-keto PGF1a	Group 1	5.2 ± 0.6	48.3 ± 4.3	19.7 ± 2.4	7.0 ± 1.4	5.5 ± 1.2	3.2 ± 0.4
(pg/.1mL)	Group 2	8.5 ± 1.2	63.6 ± 7.7	25.2 ± 2.7	13.1 ± 1.5	6.2 ± 1.0	3.7 ± 0.4
Shed Blood 6-keto PGF1a	Group 1	15.6 ± 2.0	---	30.7 ± 3.2	17.9 ± 3.6	13.4 ± 1.9	14.7 ± 1.6
(pg/.1mL)	Group 2	22.4 ± 4.9	---	37.3 ± 3.1	25.6 ± 5.2	17.6 ± 3.2	13.7 ± 1.3

BP = Cardiopulmonary Bypass BTG = beta-Thromboglobulin TXB2 = Thromboxane B2

Group 1 = Patients with isolated CABG who did not receive platelet transfusions

Group 2 = Patients with valvular disease and/or CABG who received platelet transfusions

Changes within each row were significant by repeated measure ANOVA

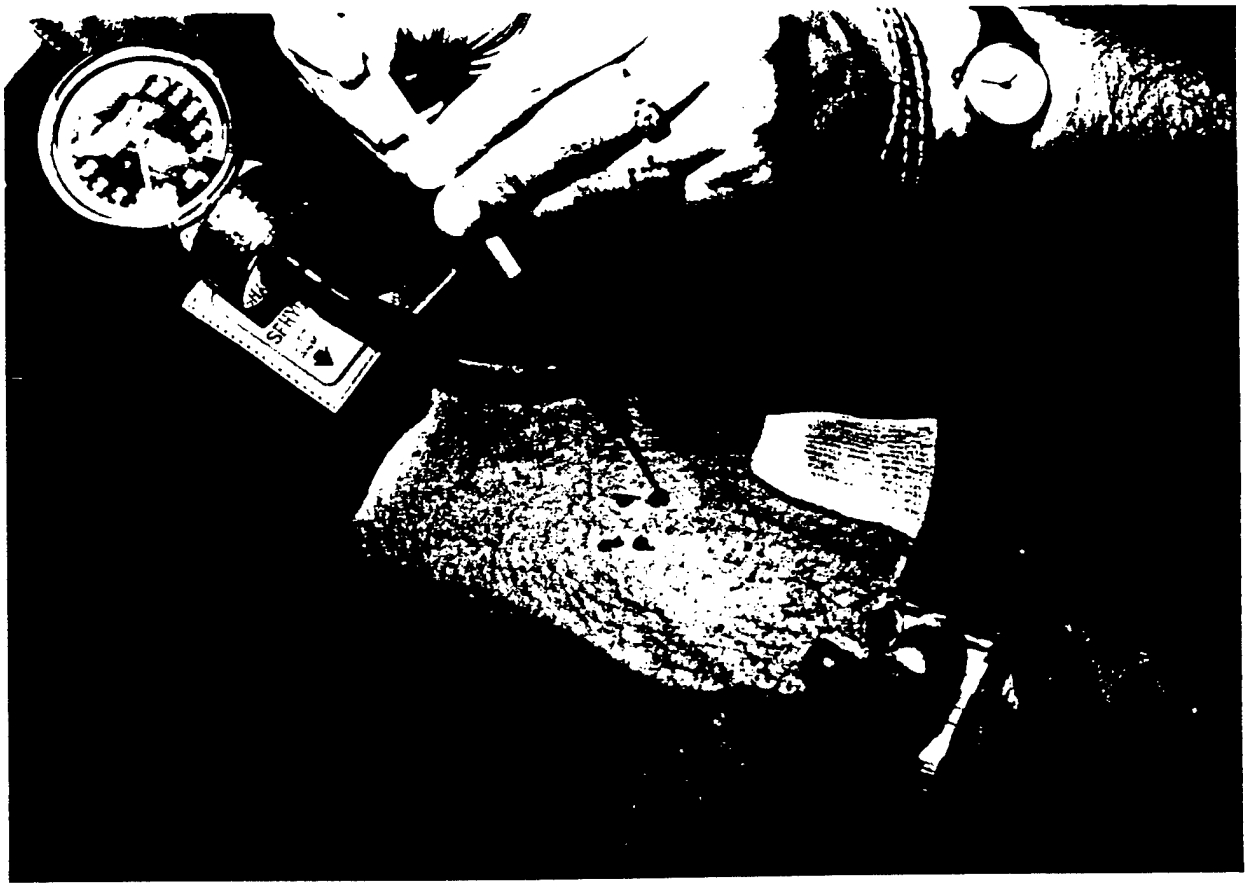

Fig 1.

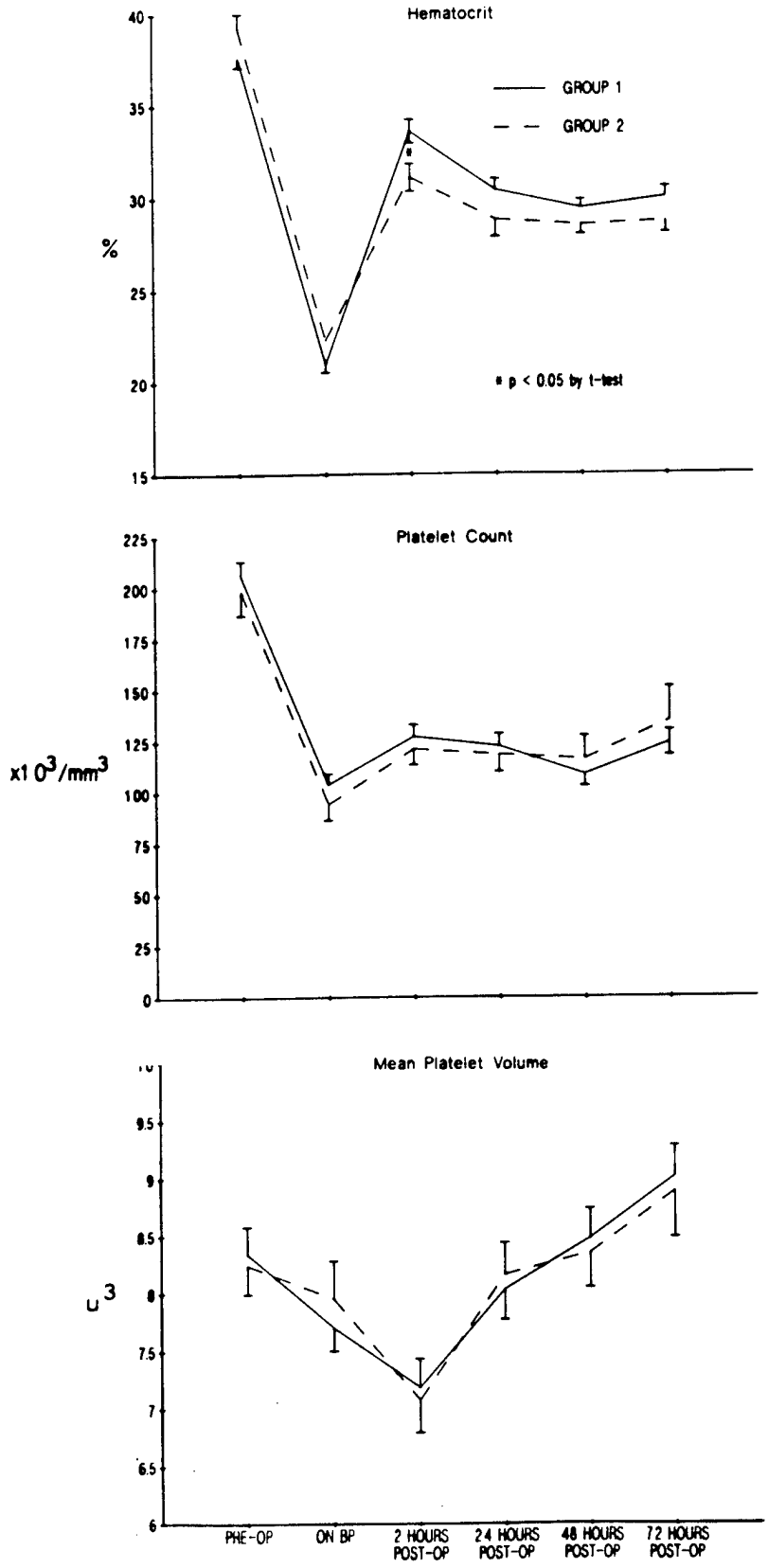
Hematocrit
GROUP 1
GROUP 2
%
40
35
30
25
20
15
* p < 0.05 by t-test
Platelet Count
x10^3/mm^3
225
200
175
150
125
100
75
50
25
0
Mean Platelet Volume
fL
9.5
9
8.5
8
7.5
7
6.5
6
PRE-OP
ON BP
2 HOURS POST-OP
24 HOURS POST-OP
48 HOURS POST-OP
72 HOURS POST-OP

Fig 2.

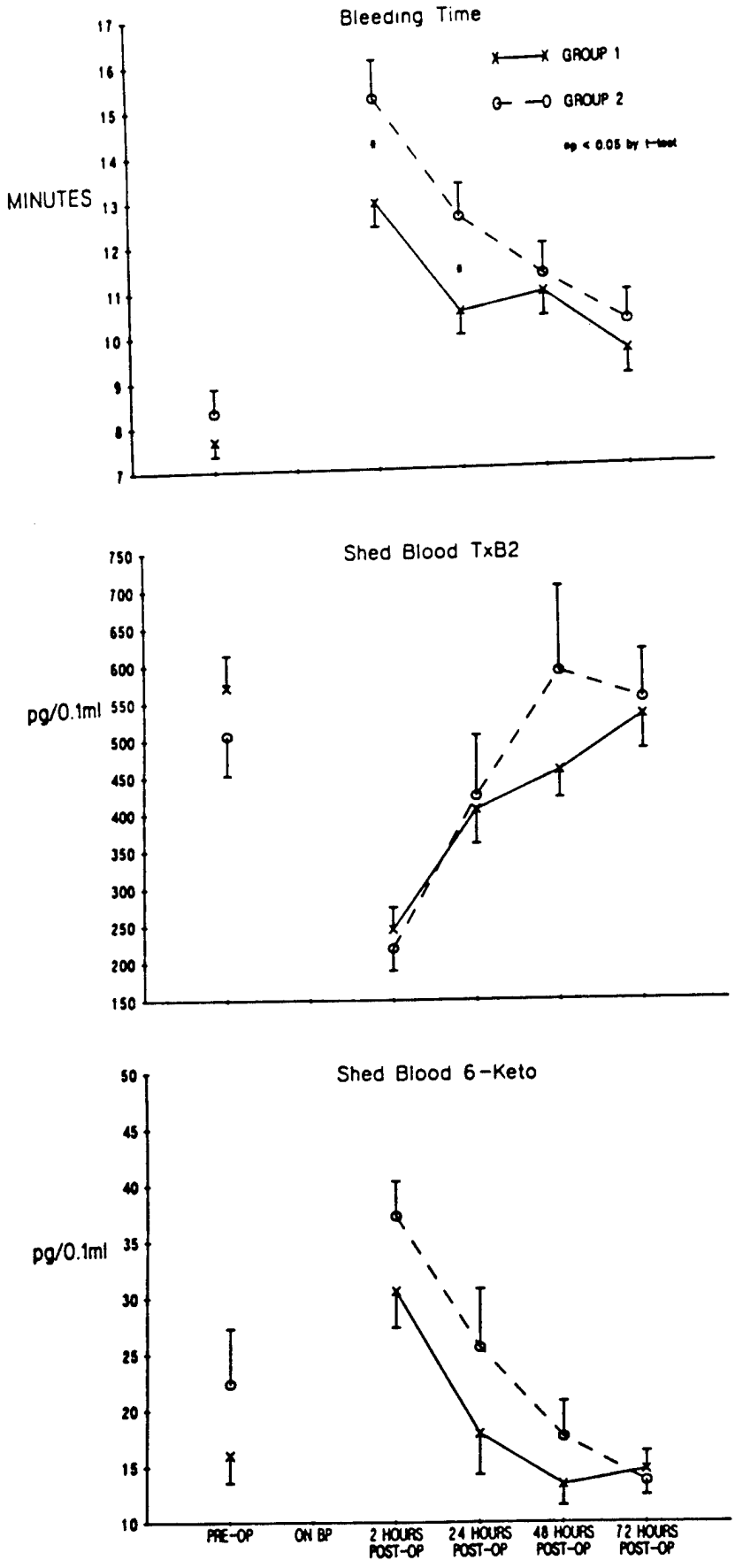
Bleeding Time
GROUP 1
GROUP 2
MINUTES
Shed Blood TxB2
pg/0.1ml
Shed Blood 6-Keto
pg/0.1ml
PRE-OP
ON BP
2 HOURS POST-OP
24 HOURS POST-OP
48 HOURS POST-OP
72 HOURS POST-OP

Fig 3.

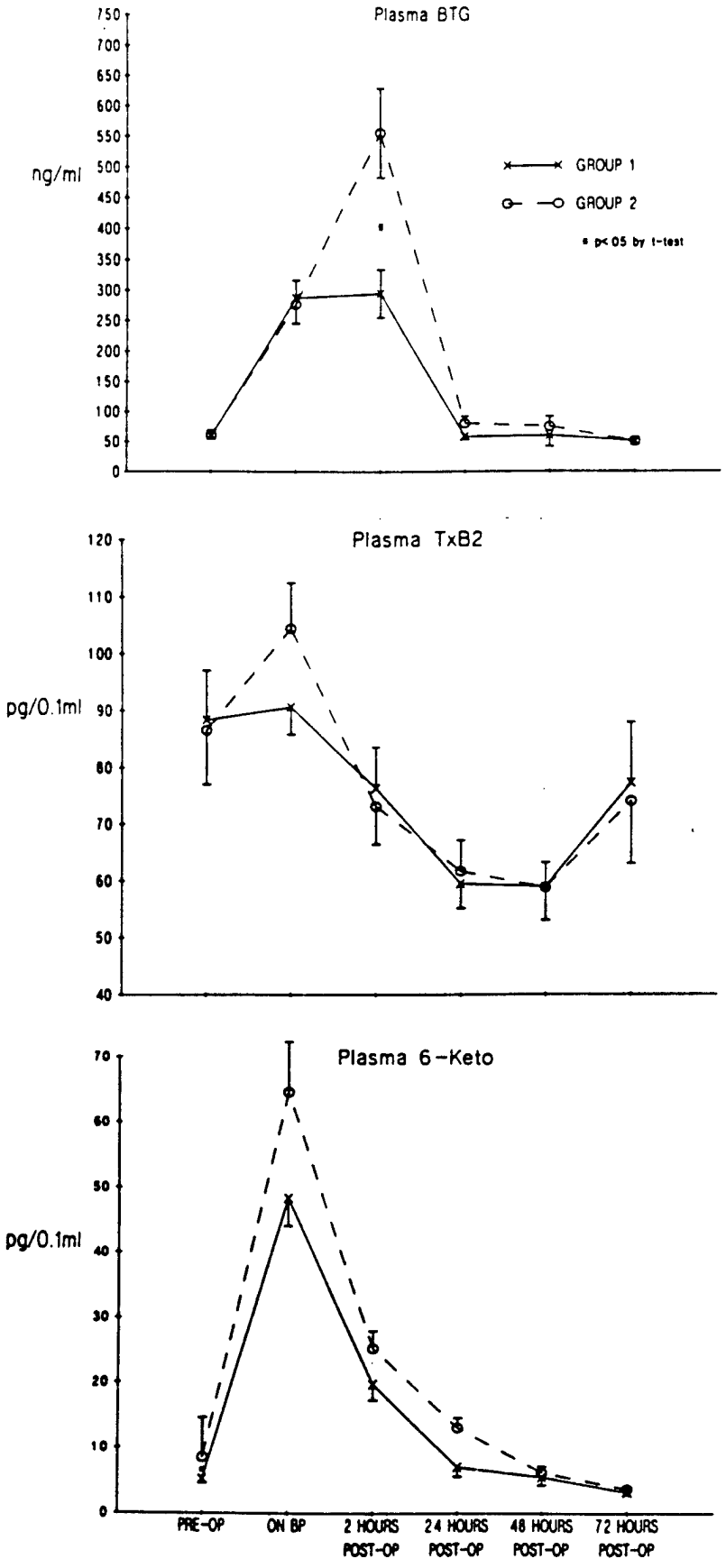
Plasma BTG
ng/ml
GROUP 1
GROUP 2
* p< 05 by t-test
Plasma TxB2
pg/0.1ml
Plasma 6-Keto
pg/0.1ml
PRE-OP
ON BP
2 HOURS POST-OP
24 HOURS POST-OP
48 HOURS POST-OP
72 HOURS POST-OP

Fig 4.

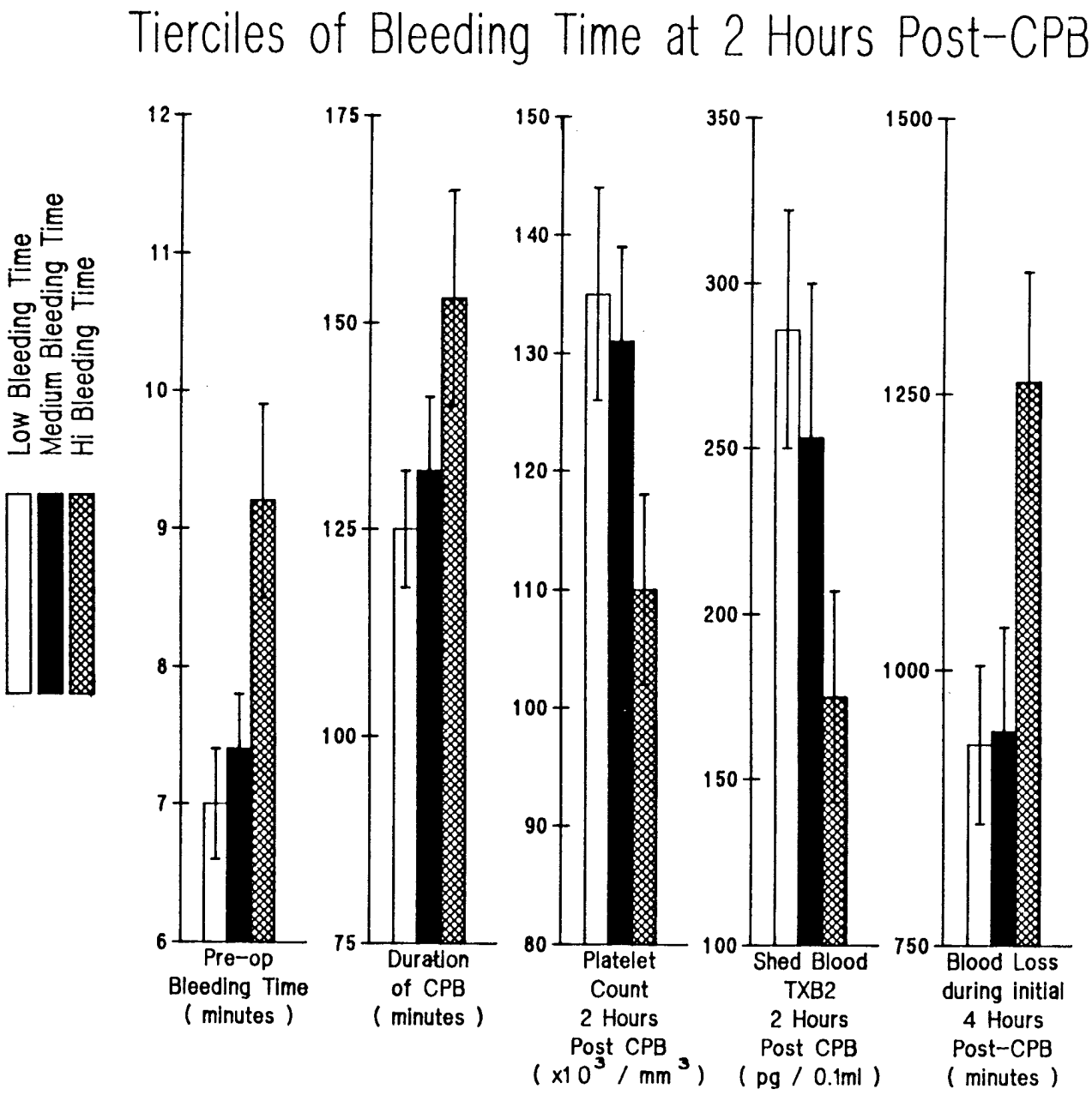
Tierciles of Bleeding Time at 2 Hours Post-CPB
Low Bleeding Time
Medium Bleeding Time
Hi Bleeding Time
12
11
10
9
8
7
6
Pre-op Bleeding Time (minutes)
175
150
125
100
75
Duration of CPB (minutes)
150
140
130
120
110
100
90
80
Platelet Count 2 Hours Post CPB (x10³ / mm³)
350
300
250
200
150
100
Shed Blood TXB2 2 Hours Post CPB (pg / 0.1ml)
1500
1250
1000
750
Blood Loss during initial 4 Hours Post-CPB (minutes)

Fig. 5

Tierciles of Total Blood Loss During the Initial 4 Hour Period Post-CPB

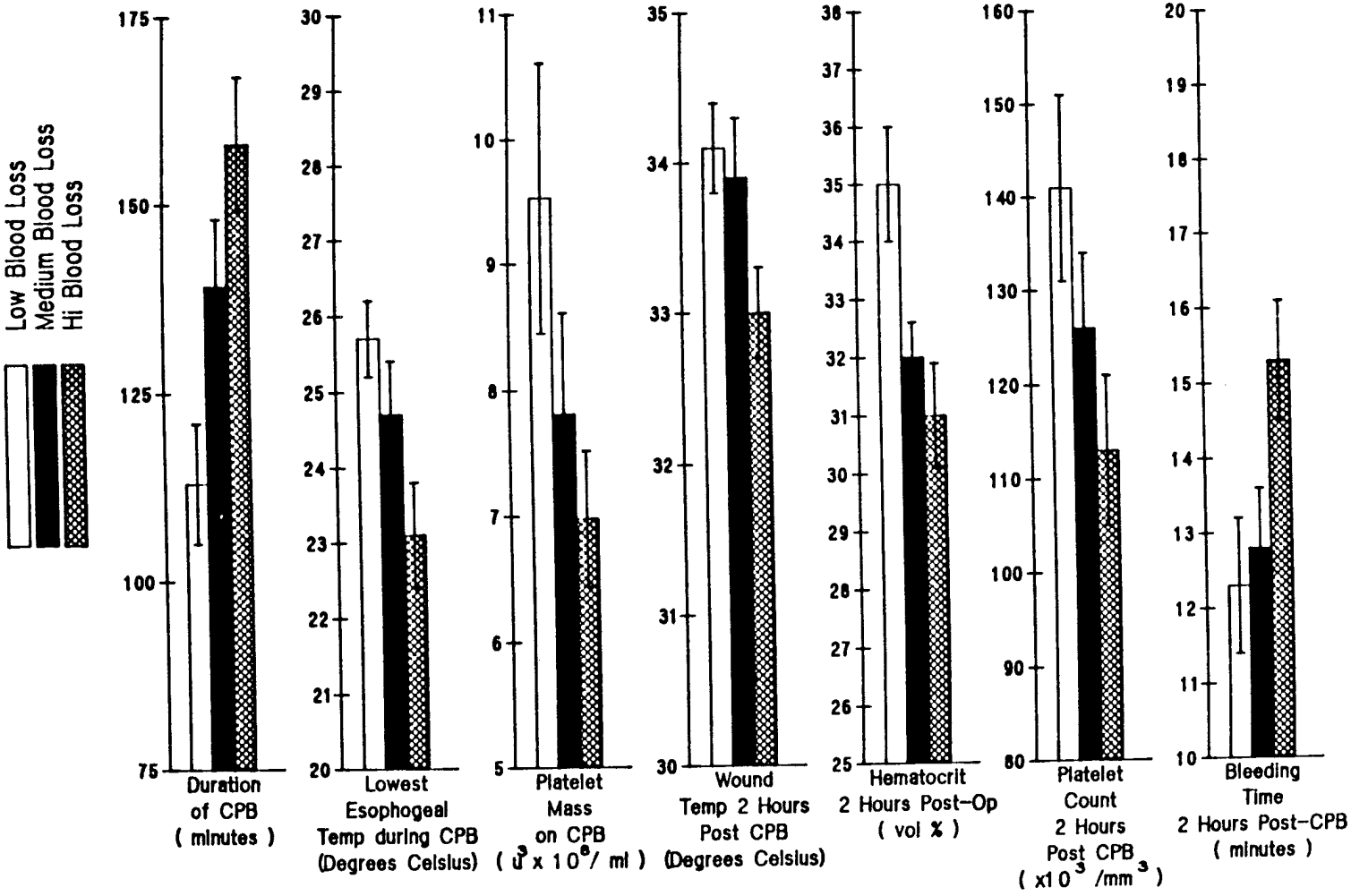

Fig. 6